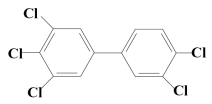
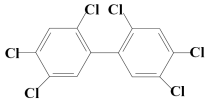
Mixture of PCB126 and PCB153



3,3',4,4',5-pentachlorobiphenyl (PCB126)



2,2',4,4',5,5"-hexachlorobiphenyl (PCB153)

Rationale for study

- Exposure-most prevalent PCB congener
- Known interactions between PCB 153 and dioxin-like compounds
  - Pharmacokinetics
  - Biochemical responses
- Unknown interaction for carcinogenicity
- TEF concept does not account for interaction between compounds with different mechanisms of action

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Study Details-PCB126:PCB153

- Female Harlan Sprague-Dawley rat only
- Oral gavage: 5 days per week
- Vehicle: corn oil:acetone (99:1) - 2.5 ml/kg
- Time points: 14-, 31-, 53- week and 2-year
- Dose groups
  - Constant ratio groups
  - "Varying ratio" groups

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Mixture study groups

PCB153 (ug/kg)	PCB 126 (ng/kg)				
	0	10	100	300	1000
0	Group 1				
10		Group 2			
100			Group 3	Group 4	
300				Group 5	
1000					Group 7
3000				Group 6	

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Constant Ratio Groups

PCB153 (ug/kg)	PCB 126 (ng/kg)				
	0	10	100	300	1000
0	Group 1				
10		Group 2			
100			Group 3	Group 4	
300				Group 5	
1000					Group 7
3000				Group 6	

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Varying Ratio Groups

PCB153 (ug/kg)	PCB 126 (ng/kg)				
	0	10	100	300	1000
0	Group 1				
10		Group 2			
100			Group 3	Group 4	
300				Group 5	
1000					Group 7
3000				Group 6	

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Mixture study design in context

PCB153 (ug/kg)	PCB 126 (ng/kg)				
	0	10	100	300	1000
0	Group 1	TR520	TR520	TR520	TR520
10	TR529	Group 2			
100	TR529		Group 3	Group 4	
300	TR529			Group 5	
1000	TR529				Group 7
3000	TR529			Group 6	

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Reporting strategy

- Carcinogenicity conclusion for the constant ratio groups
  - Groups 1,2,3,5,7
  - Focus of this presentation
- Reported significant trends across varying ratio groups
  - Groups 4,5,6
  - No carcinogenicity conclusion
- Future analyses will cross compare with PCB 153 and PCB 126 studies
  - Impact of PCB153 on RPFs for PCB126
  - Dose response for interactions

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Survival and body weight

- No effect on survival
- Decreased body weight gain
  - Groups dosed with 300 ng PCB126/kg and higher

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Biochemical effects (Constant ratio)

- Increased cytochromes P450 activity
  - Significantly increased at all doses at all time points
  - Liver CYP1A1 and CYP1A2
  - Liver CYP2B
  - Lung CYP1A2
- Alterations in thyroid hormones
  - Total and free T4: decreased at all times points
  - T3 increased: at all time points
  - TSH increased: at 14 weeks only
- Hepatocyte replication-BrdU labelling index
  - Increased at 31- and 53 weeks in highest dose group only

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Hepatic toxicity: lesion spectrum

- Increasing dose and time
  - Increasing spectrum of effects
  - Increased severity
- 14 weeks
  - Hepatocyte hypertrophy
  - Pigmentation
  - Multinucleated hepatocytes
  - Fatty change, diffuse
- 31 weeks
  - + "Toxic hepatopathy"
  - Hepatocyte replication (BrdU)
- 53 weeks
  - + Bile duct hyperplasia
  - + Oval cell hyperplasia
  - + Focal cellular alteration
  - + Cholangiofibrosis
- 2 years
  - + Nodular hyperplasia
  - + Portal fibrosis
  - + Necrosis
  - + Bile duct cysts
  - + Fatty change, focal

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Liver: Lowest affected doses (ng/kg)

Endpoint	14wk	31wk	53wk	2-year
CYP1 P450 induction	10	10	10	
Rel liver weight increase	10	10	100	
Hepatocyte BrdU labelling	NS	1000	1000	
Hepatocyte hypertrophy	10	100	300	10
Toxic hepatopathy	--	1000	1000	100
Altered hepatic foci	--	--	1000	100
Bile duct hyperplasia	--	--	1000	300
Oval cell hyperplasia	--	--	1000	100
Nodular hyperplasia	--	--	--	300
Cholangiofibrosis	--	--	NS	300
Hepatocellular adenoma	--	--	--	300
Cholangiocarcinoma	--	--	--	300

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Liver: 2 year constant ratio groups

	ng PCB126:ug PCB153/kg				
	0	10	100	300	1000
Animals per group	53	53	52	52	51
Toxic hepatopathy	0*	2	34*	48*	49*
Hepatocellular adenoma	0*	0	3 (8%)	5* (13%)	27* (68%)
Hepatocellular carcinoma [a]	0	0	0	0	2
Cholangiocarcinoma[a]	0*	0	1 (3%)	9* (24%)	30* (76%)
Hepatocholangioma[a]	0*	0	0	2 (5%)	6* (17%)

*P<0.05, [a]Historical control incidence; 0/371

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Lung: 2 year

	ng PCB126:ug PCB153/kg				
	0	10	100	300	1000
Animals per group	53	53	52	53	52
Alveolar epithelium- metaplasia, bronchiolar	0*	6*	23*	34*	32*
Squamous metaplasia	0*	0	1	2	11*
Cystic Keratinizing epithelioma[a]	0*	0	0	1 (3%)	11* (29%)
Squamous cell carcinoma[a]	0	0	0	1	1

*P<0.05, Note asterisk for controls refers to trend test.
[a]Historical control incidence; 0/371

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Oral mucosa: 2 year

	ng PCB126:ug PCB153/kg				
	0	10	100	300	1000
Animals per group	53	53	53	53	53
Gingival squamous hyperplasia	8	8	18	22	24
Gingival squamous cell carcinoma[a]	0*	0	2 (5%)	5* (13%)	9* (23%)

*P<0.05, Note asterisk for controls refers to trend test.
[a]Historical control incidence; 4/371

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Pancreas: 2 year

	ng PCB126:ug PCB153/kg				
	0	10	100	300	1000
Animals per group	53	53	52	52	50
Acinar cytoplasmic vacuolization	0*	0	0	7*	40*
Acinar atrophy	0	2	1	1	8*
Acinar adenoma/carcinoma [a]	0	1 (3%)	1 (3%)	4 (11%)	2 (6%)

*P<0.05, Note asterisk for controls refers to trend test.
[a] Historical control incidence; 1/366

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Uterus: 2 year

	ng PCB126:ug PCB153/kg				
	0	10	100	300	1000
Animals per group	53	53	53	53	53
Squamous cell carcinoma [a]	1 (3%)	1 (3%)	1 (3%)	4 (11%)	0

[a] Historical control incidence; 1/371

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Other organs: Non-neoplastic effects

- Thymic atrophy
- Thyroid follicular cell hypertrophy
- Kidney-nephropathy, pigmentation
- Adrenal cortex-atrophy
- Spleen - lymphoid follicular atrophy
- Nasal cavity
  - Respiratory epithelium- hyperplasia
  - Olfactory epithelium-metaplasia
- Forestomach-squamous hyperplasia
- Lymph node ectasia

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Conclusions- PCB126:PCB153

- Clear evidence of carcinogenicity for constant ratio mixture
- Based on
  - Cholangiocarcinoma of the liver
  - Hepatocholangioma of the liver
  - Hepatocellular neoplasms of the liver
    - Predominantly hepatocellular adenoma
    - Hepatocellular carcinoma
  - Squamous neoplasms of the lung
    - Predominantly cystic keratinizing epithelioma
    - Squamous cell carcinoma
  - Gingival squamous cell carcinoma of the oral mucosa
- Also considered to be related to treatment
  - Acinar neoplasms of the pancreas
- May have been related to treatment
  - Squamous cell carcinoma of the uterus

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Effect of increasing PCB 153 in mixture

PCB153 (ug/kg)	PCB 126 (ng/kg)				
	0	10	100	300	1000
0	Group 1	TR520	TR520	TR520	TR520
10		Group 2			
100			Group 3	Group 4	
300				Group 5	
1000					Group 7
3000				Group 6	

Varying ratio groups

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Nonneoplastic effects

- Incidence of hepatic effects increased with increasing PCB153
  - Hepatocyte hypertrophy
  - Fatty change, diffuse
  - Fatty change focal
  - Basophilic focus
  - Eosinophilic focus
  - Clear cell focus
  - Cholangiofibrosis
  - Bile duct hyperplasia
  - Liver EROD-14 weeks
- Decreased with increasing PCB153
  - Liver-EROD-53 weeks
  - Lung-Alveolar epithelium, metaplasia, bronchiolar

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Liver: Neoplasms

- Incidence increased with increasing PCB153
  - Hepatocelluar adenoma
  - Cholangiocarcinoma
  - Hepatocholangioma
- Decreased with increasing PCB153
  - Liver-PCB 126 ng/g concentration
  - Lung PCB 126 ng/g concentration
  - 31-, 53- weeks and 2 years

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Effect of PCB153

	ug PCB 153/kg + 300 ng PCB126/kg		
	100	300	3000
Animals examined	50	52	51
Hepatocellular adenoma	2 (5%)	5 (13%)	21 (50%)
Cholangiocarcinoma	7 (17%)	9 (24%)	25 (60%)
Hepatocholangioma	0	2	2
Lung CKE	1	1	1
Liver PCB126 ng/g - 2 yr	232	202	125
Lung PCB126 pg/g - 2 yr	902	459	478

Number of animals with lesion shown (survival adjusted incidence in parentheses)

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Conclusions

- Non neoplastic interactions
  - Positive effect on incidence of some hepatic non neoplastic lesions
- Effect on neoplastic incidences
  - Positive effect of PCB 153 on incidence hepatic neoplasms
- Pharmacokinetic/dynamic interaction
  - Decreased liver and lung levels at high doses of PCB153

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